The properties and distribution of inward rectifier potassium currents in pig coronary arterial smooth muscle

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1. Whole-cell potassium currents were studied in single smooth muscle cells enzymatically isolated from pig coronary arteries.

2. In cells isolated from small diameter branches of the left anterior descending coronary artery (LAD), an inward rectifier potassium current ($I_{K(IR)}$) was identified, which was inhibited by extracellular barium ions, suggesting the presence of inward rectifier potassium (K_{IR}) channels.

3. The conductance for $I_{K(IR)}$ measured in 6, 12, 60 and 140 mM extracellular potassium was a function of membrane potential and the extracellular potassium concentration.

4. On hyperpolarization, $I_{K(IR)}$ activated along an exponential time course with a time constant that was voltage dependent.

5. Inward rectifier current was compared in cells isolated from coronary vessels taken from different points along the vascular tree. Current density was greater in cells isolated from small diameter coronary arteries; at −140 mV it was $-20{\cdot}5 \pm 4{\cdot}4$ pA pF^{-1} ($n = 23$) in 4th order branches of the LAD, but $-0{\cdot}8 \pm 0{\cdot}2$ pA pF^{-1} ($n = 11$) in the LAD itself.

6. In contrast to $I_{K(IR)}$, there was little effect of arterial diameter on the density of voltage-dependent potassium current; densities at +30 mV were $12{\cdot}8 \pm 1{\cdot}3$ pA pF^{-1} ($n = 19$) in 4th order branches and $17{\cdot}4 \pm 3{\cdot}1$ pA pF^{-1} ($n = 11$) in the LAD.

7. We conclude that K_{IR} channels are present in pig coronary arteries, and that they are expressed at a higher density in small diameter arteries. The presence of an enhanced $I_{K(IR)}$ may have functional consequences for the regulation of cell membrane potential and tone in small coronary arteries.

Small arteries provide the majority of resistance to blood flow, and are therefore of central importance in the control of systemic blood pressure and local blood flow. There is increasing evidence that the responsiveness of these small arteries differs markedly from that of larger conduit arteries. For example, small coronary arteries show active responses to lumenal flow, to intravascular pressure, and to the concentration of local metabolites released from cardiac myocytes, whereas large coronary arteries are relatively insensitive to these factors (e.g. Jones, Kuo, Davis & Chilian, 1995). In many cases, the underlying basis for such functional differences between small and large arteries is unknown.

Potassium channels have diverse roles in regulating the arterial smooth muscle cell membrane potential, and therefore vascular tone (Nelson & Quayle, 1995). Large diameter coronary arteries express several types of potassium channel, including voltage-activated potassium (K_V) channels, calcium-activated potassium channels, and ATP-sensitive potassium channels (Toro, Vaca & Stefani, 1991; Volk, Matsuda & Shibata, 1991; Ishikawa, Hume & Keef, 1993; Dart & Standen, 1993). More recently, preliminary reports have identified inward rectifier potassium (K_{IR}) channels in small diameter coronary arteries or arterioles (Bonev, Robertson & Nelson, 1994; Klieber & Daut, 1994; Quayle, Dart & Standen, 1995).

In small cerebral and mesenteric arteries inward rectifier potassium ($I_{K(IR)}$) currents provide the dominant potassium conductance around the resting membrane potential (Hirst, Silverberg & van Helden, 1986; Edwards & Hirst, 1988; Edwards, Hirst & Silverberg, 1988; Quayle, McCarron, Brayden & Nelson, 1993). Activation of K_{IR} channels underlies the dilatation of small arteries, which occurs when the extracellular potassium concentration ($[K^+]_o$) is raised from 5 to 10 mM (Edwards *et al.* 1988; McCarron & Halpern, 1990). A mismatch between metabolic demand and blood supply elevates extracellular potassium, and the potassium-induced dilatation may be partly responsible for metabolic vasodilatation in some vascular beds, including the coronary circulation (Scott, Frohlich, Hardin & Haddy, 1961; Olsson & Bünger, 1987). Extracellular potassium accumulation also has pathological implications in the heart, being a major

factor in the generation of anginal pain and of ischaemia-related cardiac arrhythmias (Wilde & Asknes, 1995).

K_{IR} channels in coronary arteries may therefore have a role in the metabolic regulation of cardiac blood flow, in potassium homeostasis in the heart, and in controlling the smooth muscle cell membrane potential. Despite this, little is known about the K_{IR} channel in coronary arteries. One reason for this is that K_{IR} channels do not appear to provide a prominent potassium conductance in single smooth muscle cells isolated from large vessels, which are generally used for arterial electrophysiology (Nelson & Quayle, 1995). In this study we have identified $I_{K(IR)}$ currents in single smooth muscle cells isolated from pig coronary arteries. We have characterized these currents, often using solutions with high $[K^+]_o$ to increase their size and so make them easier to study. We have also studied the distribution of K_{IR} channels within the coronary vascular bed, and find that $I_{K(IR)}$ is at a higher density in cells from smaller arteries. This distribution of the K_{IR} channel in the coronary vasculature is discussed in relation to the possible physiological roles of this channel.

METHODS

Dissection and cell isolation

Coronary arteries were dissected from pig hearts, which were obtained from a local abattoir. The left anterior descending coronary artery (LAD) was located and this, or 1st, 2nd, 3rd or 4th order side branches of this artery, were dissected. Branch order was defined according to the convention that, on bifurcation of an artery, the smaller branch was taken as the higher number order. Arteries were dissected in cold saline solution containing (mM): 137 NaCl, 5·4 KCl, 0·44 NaH_2PO_4, 0·42 Na_2HPO_4, 1 $MgCl_2$, 2 $CaCl_2$, 10 Hepes, 10 glucose; pH readjusted to 7·4 with NaOH. The diameter of the freshly dissected artery was measured using either an eye-piece graticule or a video dimension analyser (Living Systems Instruments, Burlington, VT, USA). Single cells were obtained using an enzymatic digestion procedure. Arteries were incubated for about 35 min at 35 °C in a 0·1 mM Ca^{2+} saline solution containing approximately 1·5 mg ml^{-1} papain and 1 mg ml^{-1} dithioerythritol. The artery was then transferred into a 0·1 mM Ca^{2+} saline solution containing approximately 1·5 mg ml^{-1} collagenase and 1 mg ml^{-1} hyaluronidase for about 20 min. For some experiments, cells were isolated using a collagenase and elastase digestion medium (see Dart & Standen, 1995). Similar results were obtained for both cell isolation procedures, and have therefore been pooled. Enzyme incubation time was the same for all branch orders. After enzyme treatment the artery was rinsed in 0·1 mM Ca^{2+} saline, and single cells were dispersed in this solution by trituration of the tissue through a heat-polished Pasteur pipette. Cells were stored in the refrigerator and used on the day of preparation.

Data recording and analysis

Whole-cell currents were recorded from single smooth muscle cells using the patch clamp technique (Hamill, Marty, Neher, Sakmann & Sigworth, 1981). Membrane current was recorded, and membrane voltage controlled, using pCLAMP 5, an Axolab interface and an Axopatch 200 amplifier (Axon Instruments). Data were recorded on computer for later analysis using pCLAMP 5 or software written by Dr Noel Davies (Davies, 1993). Membrane current was recorded in the conventional whole-cell configuration or in the perforated patch configuration using amphotericin B (Horn & Marty, 1988). Patch pipettes were made from thin-walled borosilicate glass (Clark Electromedical, Pangbourne, Berks, UK, or Garner Glass, Claremont, CA, USA). Electrodes were coated with sticky wax to reduce capacitance (Kemdent, Swindon, Wilts, UK). Currents were filtered at 5 kHz, except where indicated. Electrode resistance prior to sealing was about 5 MΩ, and after sealing was >10 GΩ. Experiments were conducted at room temperature (18–22 °C), and results are expressed as means ± S.E.M.

For studies on channel distribution, the current which was sensitive to inhibition by 0·5 mM barium was measured at a membrane potential of −140 mV. Barium ions inhibit inward rectifier potassium channels in arterial smooth muscle in the 1–10 μM concentration range (Quayle *et al.* 1993; see also Figs 1 and 7). A membrane potential of −140 mV is considerably negative to the half-activation potential of both voltage-activated and calcium-activated potassium channels in smooth muscle (Nelson & Quayle, 1995). The contribution of calcium-activated potassium channels and ATP-sensitive potassium channels to measured current was also minimized by using a low $[Ca^{2+}]_o$ of 0·1 mM and by inclusion of EGTA and ATP in the pipette solution (see below).

Solutions

For conventional whole-cell recordings, the intracellular solution contained (mM): 107 KCl, 33 KOH, 10 Hepes, 5 EGTA, 5 EDTA, 3 Na_2ATP; pH 7·2. The 140 mM K^+ extracellular solution contained (mM): 140 KCl, 1 $MgCl_2$, 0·1 $CaCl_2$, 10 Hepes–NaOH, 10 glucose; pH 7·4. K^+ extracellular solutions (60, 12 and 6 mM) were made by substituting the appropriate amount of NaCl for KCl in the above solution. For perforated patch recordings, the intracellular solution contained (mM): 143 KCl, 1 $MgCl_2$, 0·5 EGTA, 10 Hepes; pH 7·2. Amphotericin B was made as a 6 mg ml^{-1} stock solution in dimethylsulphoxide and stored at −20 °C. Amphotericin B stock solution (20 μl) was added to 5 ml of pipette solution prior to use, to give a final concentration of 240 μg ml^{-1}. The extracellular calcium concentration was 1·8 mM for perforated patch whole-cell recordings. Chemicals and enzymes for cell isolation were purchased from Sigma.

Junction potential

The calculated junction potential between the pipette solution used for whole-cell recording and the 6 mM K^+ bath solution in which seals were made was +4 mV. We have not corrected for this junction potential, which accounts for the approximate 4 mV discrepancy between the reversal potential for $I_{K(IR)}$ and the calculated potassium equilibrium potential (E_K) in our current–voltage relationships (e.g. Figs 1*B* and 2*A*).

RESULTS

Inward rectifier potassium currents in pig coronary arteries

Figure 1*A* illustrates $I_{K(IR)}$ currents of small coronary arteries from the pig. Currents were recorded from a single smooth muscle cell using the conventional whole-cell clamp technique and in response to voltage steps from a holding potential of 0 mV to test potentials between −90 and +40 mV in 10 mV increments. Both extracellular ($[K^+]_o$) and intracellular ($[K^+]_i$) potassium concentrations were 140 mM. The holding potential of 0 mV will cause substantial inactivation of voltage-dependent potassium

currents, and experimental solutions were designed to minimize the contribution of potassium currents other than the inward rectifier to membrane current (see Methods and Volk *et al.* 1991). Under these conditions, substantial inward currents were recorded at membrane potentials negative to the E_K of 0 mV, while much smaller outward currents were recorded positive to E_K. The current–voltage relationship for this cell is illustrated in Fig. 1*B*, and shows pronounced inward rectification.

An alternative approach to recording $I_{K(IR)}$ makes use of the sensitivity of this channel to inhibition by extracellular barium ions (Standen & Stanfield, 1978; Quayle *et al.* 1993). Whole-cell current was recorded in response to a voltage step from −60 to −140 mV for 20 ms, followed by a depolarizing voltage ramp at 1 mV ms^{-1} from this potential to +50 mV (Fig. 1*C*). Under control conditions, an inward current was present at membrane potentials negative to E_K. At potentials positive to E_K an outward current developed, and this is attributable mainly to voltage-activated potassium channels (Quayle *et al.* 1993; see below). When the cell was superfused with an extracellular solution containing 0·5 mM barium, the inward current was substantially reduced (Fig. 1*C*). In contrast, barium had little effect on outward current, so that the current traces in control and in barium-containing solution are nearly superimposed at membrane potentials positive to E_K. Barium ions inhibit K_{IR} channels in cerebral artery smooth muscle cells at this concentration (Quayle *et al.* 1993). The barium-sensitive difference current was obtained by subtracting the current in the presence of 0·5 mM barium from that in control solution (Fig. 1*C*). As expected for an inward rectifier, the current is larger in the inward direction than in the outward direction, and reverses close to the E_K of 0 mV.

Effect of changing the extracellular potassium concentration on inward rectifier currents

The potassium selectivity of $I_{K(IR)}$ was confirmed by changing the extracellular potassium concentration and recording barium-sensitive membrane currents in response to a voltage ramp protocol similar to that shown in Fig. 1*C*. When $[K^+]_o$ was reduced from 140 to 60 mM and then to

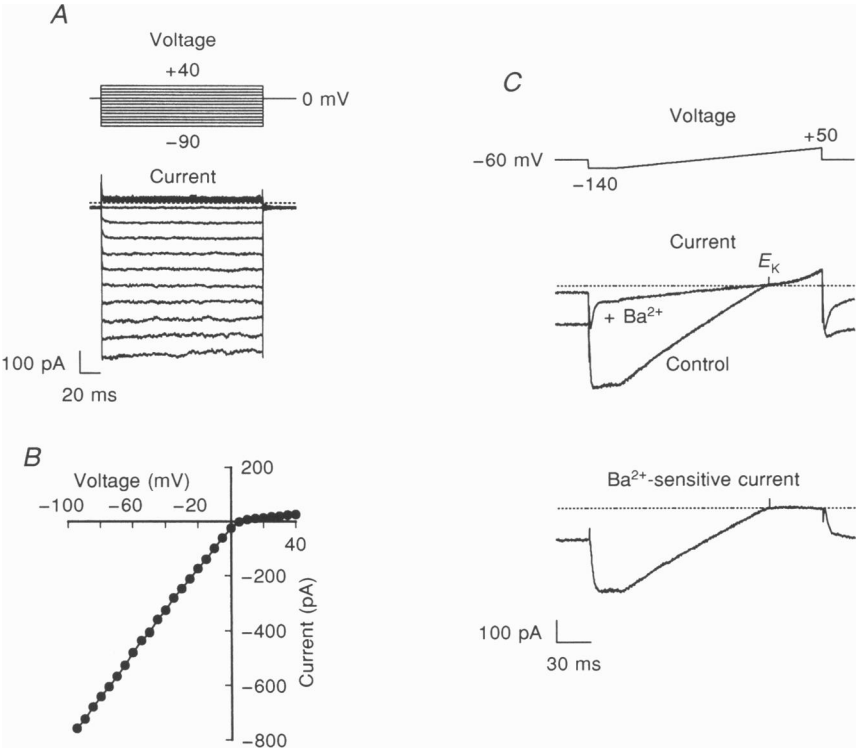

Figure 1. Inward rectifier potassium currents in pig coronary artery smooth muscle cells

A, whole-cell potassium currents recorded in response to voltage steps from a holding potential of 0 mV to test potentials between −90 and +40 mV in 10 mV increments. The upper panel illustrates the voltage protocol and the lower panel the current recordings. $[K^+]_o$ and $[K^+]_i$ were 140 mM. The dashed line indicates the zero current level. *B*, current–voltage relationship for the cell illustrated in *A*, with voltages spaced in 5 mV increments. Currents were measured as the mean current between 110 and 150 ms from the start of the voltage step. *C*, whole-cell currents recorded in response to a voltage ramp from −140 to +50 mV in symmetrical $[K^+]$ of 140 mM. The upper panel shows the voltage protocol, the centre panel the currents recorded in the absence (Control) and presence (+ Ba^{2+}) of 0·5 mM barium in the extracellular solution, and the lower panel shows the barium-sensitive current obtained as the difference between the two current records. The dashed line shows the zero current level and E_K is the potassium equilibrium potential.

6 mM, changing the E_K from 0 to −21·4 and −79·6 mV, respectively, the reversal potential of the barium-sensitive current shifted towards the new E_K, as expected for a potassium-selective channel (Fig. 2*A*). It is also clear from Fig. 2*A* that the maximal conductance at voltages negative to E_K is lower in low $[K^+]_o$. We measured this maximal conductance as the slope conductance 20 mV negative to E_K, and its dependence on $[K^+]_o$ is shown in Fig. 2*B*. The experimental results are very well fitted by a line drawn to the expression:

$$\text{Maximal slope conductance} = c([K^+]_o)^n, \quad (1)$$

with values of the constants c and n of 0·145 nS and 0·5, respectively. It is likely that this dependence of maximal slope conductance on the square root of $[K^+]_o$ results from the change in single channel conductance, since, in K_{IR} channels of cardiac myocytes, the unitary conductance shows a very similar relationship to $[K^+]_o$ (Sakmann & Trube, 1984).

Figure 2*A* also shows that barium-sensitive outward currents remained small at membrane potentials positive to E_K, indicating that the voltage dependence of $I_{K(IR)}$ conductance shifts with $[K^+]_o$. Similar results were seen in five other cells. This effect, and the dependence of maximal conductance on $[K^+]_o$, are characteristic features of K_{IR} channels, including those expressed from the mouse IRK1 gene (e.g. Hagiwara & Yoshii, 1979; Stanfield *et al.* 1994). This contrasts with the behaviour of other smooth muscle potassium channels, where the voltage range for activation does not depend on the driving force on potassium ions, and further confirms the nature of the current as $I_{K(IR)}$. Figure 3*A* shows the membrane current recorded at membrane potentials around the E_K with 60 and 140 mM extracellular potassium (K_o^+), displayed at higher gain. This figure illustrates two additional features characteristic of $I_{K(IR)}$ currents. First, the potassium current is small at membrane potentials positive to E_K and declines with membrane depolarization positive to E_K, so that the current–voltage relationship shows a region of negative slope conductance. Secondly, on increasing $[K^+]_o$ from 60 to 140 mM there is a voltage range where outward potassium current is greater with 140 mM K_o^+, e.g. compare the currents at around +20 mV in Fig. 3*A*. This cross-over of the current–voltage relationships on increasing $[K^+]_o$ is a feature of $I_{K(IR)}$ (Hille & Schwarz, 1978; Hagiwara & Yoshii, 1979), and can also be detected, though with more difficulty, in the physiological range of $[K^+]_o$ (see Fig. 4).

The effect of changing $[K^+]_o$ on the voltage dependence of gating of $I_{K(IR)}$ was investigated by measuring the chord conductance and is illustrated in Fig. 3*B*. Chord conductance (g_K) was calculated as $g_K = I/(V - V_{rev})$, where I is the membrane current; V, the membrane potential; and V_{rev}, the reversal potential of the current. The conductance is a function of both membrane potential and of $[K^+]_o$. The voltage dependence of the conductance can be described by a Boltzmann relationship:

$$g_K/g_{K,max} = \{1 + \exp[(V - V_{0.5})/k]\}^{-1}, \quad (2)$$

with a steepness factor, k, in this cell of 6·0 mV and a mid-point, $V_{0.5}$, of +0·9 mV with 140 mM K_o^+. When $[K^+]_o$ was reduced to 60 mM, changing the E_K by −21·4 mV, the mid-point of the conductance–voltage curve shifted by −20·1 mV. In similar experiments on a number of cells the mid-point shifted from −4·8 ± 1·9 mV in 140 mM K_o^+ ($n = 6$) to −20·7 ± 1·1 mV in 60 mM K_o^+ ($n = 5$). Such a shift of the voltage dependence of channel gating with $[K^+]_o$ is a further characteristic property of K_{IR} channels. The steepness of the conductance–voltage relationship did not differ significantly with a change in $[K^+]_o$ from 60 to 140 mM; the mean value of k was 7·4 ± 0·6 mV ($n = 10$ cells), corresponding to an equivalent gating charge of 3·4.

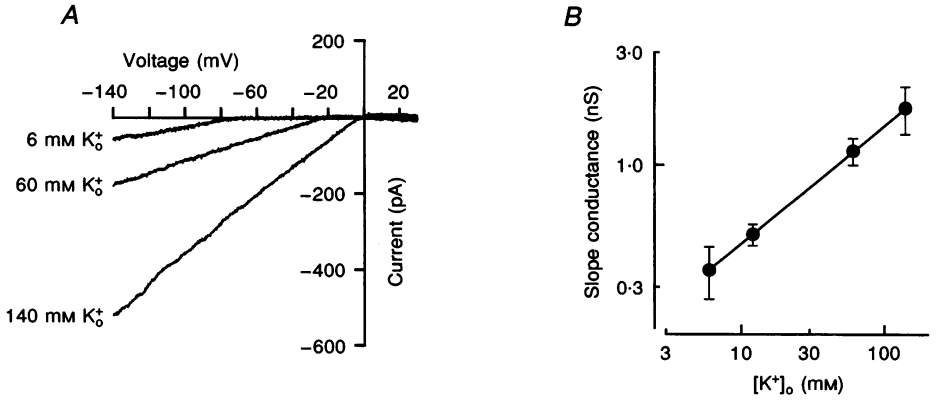

Figure 2. Potassium dependence of the inward rectifier current and conductance

A, barium-sensitive whole-cell current recorded from a cell with a $[K^+]_i$ of 140 mM and $[K^+]_o$ of 6, 60 and 140 mM, as indicated. *B*, dependence of the maximal slope conductance of $I_{K(IR)}$, measured as described in the text, on $[K^+]_o$. The points show mean values ± S.E.M. from 4 cells exposed to $[K^+]_o$ of 6, 12, 60 and 140 mM, and the line is the best fit to eqn (1).

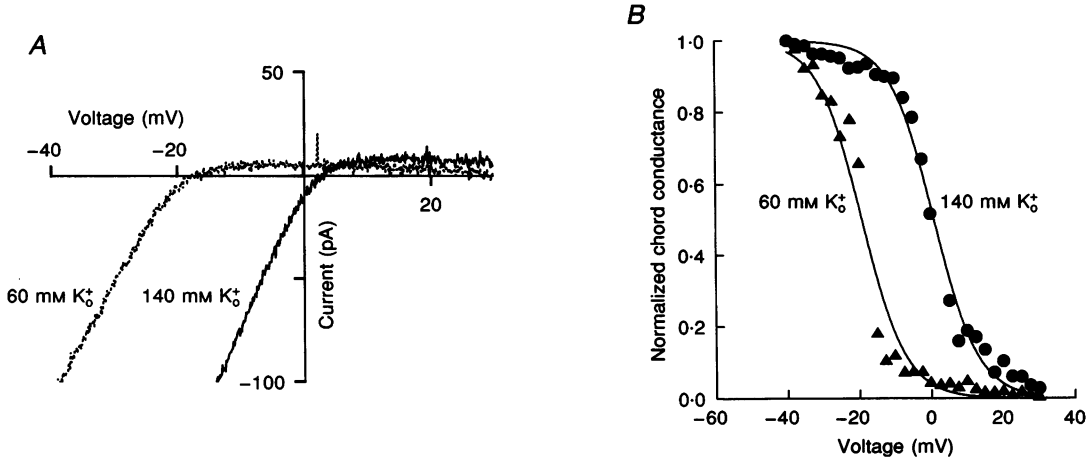

Figure 3. Conductance–voltage relationships for the inward rectifier

A, $I_{K(IR)}$ currents recorded under the same conditions as those of Fig. 2*A* with $[K^+]_o$ of 60 and 140 mM, but in a different cell and at a higher resolution, illustrating the reversal potential of the current and outward currents through the K_{IR} channel. Reversal potentials in both solutions lie about 4 mV positive to the calculated E_K because of the junction potential present before seal formation (see Methods). *B*, normalized chord conductance for the barium-sensitive current illustrated in Fig. 2*B* plotted against membrane potential in 60 (▲) and 140 mM (●) K_o^+. The continuous lines are Boltzmann relationships drawn using eqn (2) with a k value of 6·03 mV and mid-points of −19·2 mV in 60 mM K_o^+ and +0·9 mV in 140 mM K_o^+.

The low conductance of the inward rectifier in the physiological $[K^+]_o$ of 6 mM makes outward currents hard to resolve in many cells. Nevertheless, this is sometimes possible, and we show an example in Fig. 4*A*. The records have been digitally filtered at 160 Hz to make them easier to distinguish, and it can be seen that doubling $[K^+]_o$ to 12 mM shifted the reversal potential as expected, and that there is a corresponding shift in the conductance–voltage relationship of Fig. 4*B*. The membrane potential of smooth muscle cells in arteries at physiological pressures is in the range −40 to −50 mV, considerably positive to E_K (Harder, 1984; Nelson, Patlak, Worley & Standen, 1990). At such

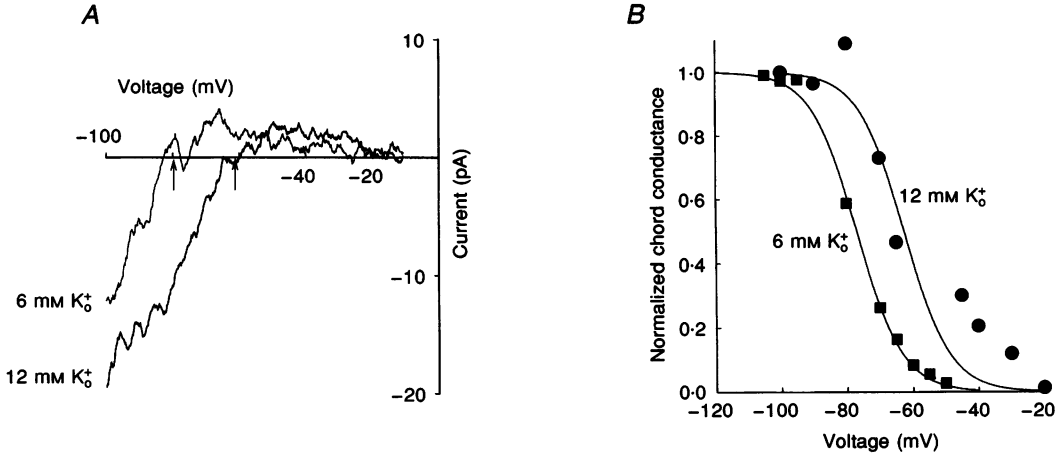

Figure 4. Inward rectifier currents in 6 and 12 mM K_o^+

A, barium-sensitive whole-cell current in the voltage range around the reversal potential recorded from a cell with a $[K^+]_i$ of 140 mM and $[K^+]_o$ of 6 and 12 mM. The current scale is expanded to show the small outward currents, and the arrows indicate the calculated values of E_K in 6 and 12 mM K_o^+ (−79·6 and −62·1 mV, respectively). *B*, normalized chord conductance for the barium-sensitive current illustrated in *A* plotted against membrane potential in 6 (■) and 12 mM (●) K_o^+. The continuous lines are Boltzmann relationships drawn using eqn (2) with a k value of 6·89 mV and mid-points of −77·0 mV in 6 mM K_o^+ and −62·5 mV in 12 mM K_o^+.

membrane potentials, the results in Fig. 4*A* suggest that increasing $[K^+]_o$ to 12 mM should lead to an increase in outward current through K_{IR} channels.

The time course of activation

When a hyperpolarizing voltage step was applied to a coronary cell from a holding potential of 0 mV (close to E_K, with $[K^+]_o = 140$ mM), $I_{K(IR)}$ showed an instantaneous increase followed by activation along an exponential time course (Fig. 5*A*). This behaviour is characteristic of inward rectifiers, and recent work on cloned K_{IR} channels by Lopatin, Makhina & Nichols (1995) suggests that the initial step represents relief of block by putrescine and Mg^{2+}, while the exponential phase results from the reversal of block by spermine and spermidine. Figure 5*A* shows that the rate of the exponential activation increased with hyperpolarization. The mean value for the time constant of the exponential fitted to the current activation was 2·71 ± 0·22 ms at −10 mV, 1·55 ± 0·12 ms at −20 mV, 0·80 ± 0·10 ms at −30 mV, and 0·57 ± 0·05 ms at −40 mV ($n = 8$ cells). The time constant for activation was an exponential function of membrane potential (Fig. 5*B*). The line fitted to the data is given by the equation:

$$\tau(V) = \tau(0)\exp(V/s), \quad (3)$$

where $\tau(0)$, the value of the time constant at 0 mV, was 4·8 ms and s, a factor describing the voltage sensitivity of the time constant, was 16·1 mV. In four cells studied in 60 mM K_o^+, the relationship between τ and voltage was shifted by −20 mV, close to the shift in E_K, so that the fitted $\tau(0)$ was 19 ms, while the voltage sensitivity appeared unchanged ($s = 16{\cdot}7$ mV; Fig. 5*B*).

The dependence of inward rectifier current density on arterial diameter

Previous studies of potassium currents in coronary artery smooth muscle cells isolated from relatively large coronary arteries have not identified substantial inward potassium currents under similar experimental conditions to those used here (e.g. Dart & Standen, 1993; Xu & Lee, 1994). This raises the possibility that the $I_{K(IR)}$ current may be located preferentially in small coronary arteries. Further experiments were therefore designed to investigate the effect of arterial diameter on $I_{K(IR)}$ current density. For these studies the conventional whole-cell clamp was used. This allowed us to minimize the contribution of other potassium channels in our recordings (see Methods), and to control some variables which may affect the amplitude of $I_{K(IR)}$, e.g. the intracellular calcium and magnesium concentrations. Figure 6*A* and *B* illustrates the effect of 0·5 mM extracellular barium on potassium currents in a cell isolated from the LAD (outer diameter = 1852 μm), and in a cell isolated from a 4th order branch of this artery (outer diameter = 130 μm). Current was recorded in response to a depolarizing voltage ramp from −140 to +50 mV under control conditions (140 mM K_o^+, 140 mM K_i^+), and after application of 0·5 mM extracellular barium. In contrast to cells isolated from smaller vessels, little inward potassium current was present in cells isolated from larger arteries, and barium had limited effect on potassium currents in these cells.

The effect of arterial diameter on $I_{K(IR)}$ is summarized in Fig. 6*C*. $I_{K(IR)}$ current density, measured as the current at −140 mV which was inhibited by 0·5 mM barium, is plotted against the external diameter of the artery from which the

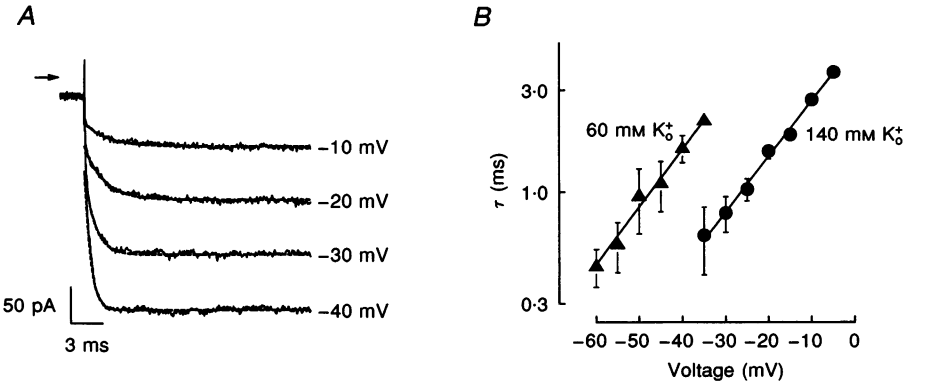

Figure 5. Time-dependent activation of inward rectifier current on membrane hyperpolarization

A, $I_{K(IR)}$ currents recorded in response to a hyperpolarizing voltage step from a holding potential of 0 mV to −10, −20, −30 or −40 mV as indicated. Dashed lines are fits to single exponentials with time constants of 2·00 ms at −10 mV, 1·42 ms at −20 mV, 0·88 ms at −30 mV and 0·49 ms at −40 mV. $[K^+]_o$ and $[K^+]_i$ were both 140 mM. Since the lines fit the experimental records rather well, they are hidden by the recording in some places. The arrow indicates the zero current level. *B*, voltage dependence of the time constant of channel activation (τ) for cells in 60 (▲) and 140 mM (●) K_o^+. The points show mean values ± S.E.M. (where larger than the symbol) for 4 and 8 cells, respectively, while the best fit lines are drawn to eqn (3) with the parameters described in the text.

cell was isolated. The current is normalized to cell capacitance and each data point represents an individual cell. There is an increased density of $I_{K(IR)}$ current in cells isolated from smaller coronary arteries. For example, in 4th order branches of the LAD (outer diameter, 93–290 μm) there was $-20{\cdot}5 \pm 4{\cdot}4$ pA pF^{-1} (23 cells) of $I_{K(IR)}$ at -140 mV (140 mM K_o^+, 140 mM K_i^+; assessed as the 0·5 mM barium-sensitive current). In comparison, in cells isolated from the LAD (outer diameter, 1850–2520 μm), there was $-0{\cdot}8 \pm 0{\cdot}2$ pA pF^{-1} (11 cells) of $I_{K(IR)}$ ($P < 0{\cdot}01$, Student's t test). Although $I_{K(IR)}$ currents in Fig. 6 are normalized for cell capacitance, this did not vary significantly with the size of the source artery. For example, cells from the LAD had a cell capacitance of $19{\cdot}8 \pm 2{\cdot}1$ pF ($n = 11$), while cells from 4th order branches had a capacitance of $19{\cdot}0 \pm 0{\cdot}9$ pF ($n = 23$).

Inward rectifier currents in the perforated patch whole-cell configuration

The difference in $I_{K(IR)}$ current density that we observed between cells from small and large arteries was also seen for currents recorded using the perforated patch whole-cell clamp technique (Horn & Marty, 1988). In this recording configuration, electrical access to the cell interior was

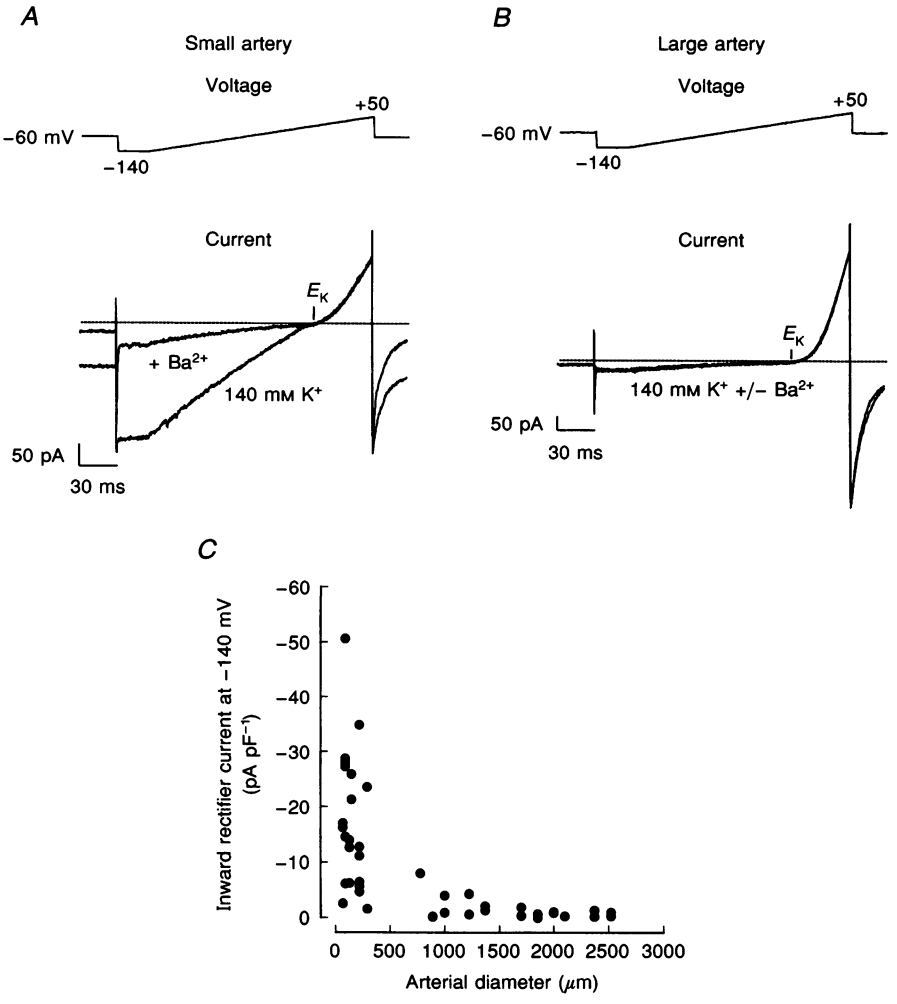

Figure 6. Effect of arterial diameter on the density of inward rectifier potassium current in single cells from pig coronary arteries

A, whole-cell potassium currents recorded from a cell isolated from a small diameter coronary artery (130 μm). Conventional whole-cell recording, with $[K^+]_o$ and $[K^+]_i$ of 140 mM. Current was recorded in response to a voltage ramp from -140 to $+50$ mV in control solution and in the presence of 0·5 mM barium. Inward current was substantially reduced by barium while outward currents recorded under the two conditions are virtually superimposed. *B*, current recorded in a cell isolated from a large diameter coronary artery (1852 μm) under the same experimental conditions as in *A*. Currents in the absence and presence of barium are virtually identical in this cell. *C*, density of $I_{K(IR)}$ current as a function of the external diameter of the artery from which single smooth muscle cells were isolated. Current is normalized to cell capacitance and is the 0·5 mM barium-sensitive current measured at -140 mV. Each symbol represents an individual cell.

obtained by the pore-forming antibiotic amphotericin B, thus minimizing disruption of the cell cytoplasm. These experiments were performed in the course of a separate study designed primarily to investigate effects of hypoxia on ATP-sensitive ($I_{K(ATP)}$) currents, so that detailed experimental conditions were somewhat different from those used in the conventional whole-cell experiments described in the rest of this paper. The whole-cell current recorded in response to a voltage ramp from −100 to +40 mV in a cell isolated from a small diameter artery is shown in Fig. 7*A*. Both $[K^+]_o$ and $[K^+]_i$ were 140 mM. Under control conditions, an inward current was present at membrane potentials negative to the E_K of 0 mV. At potentials positive to E_K an outward current developed. This outward current is primarily through voltage-activated potassium channels (Quayle *et al.* 1993). An extracellular barium concentration of 30 μM substantially reduced inward current and had little effect on outward current. These results are qualitatively similar to those obtained in conventional whole-cell recordings (Fig. 1). The barium-sensitive current–voltage relationship illustrated in Fig. 7*B* was obtained by subtracting the current in the presence of 30 μM barium from that in control solution. Inward current is present at higher density in small coronary arteries in comparison with large coronary arteries, as in conventional whole-cell recordings (Fig. 7*C*).

Although the same trend with arterial diameter was seen using perforated patch recording, it is clear that the magnitude of $I_{K(IR)}$ was much smaller than in conventional whole-cell recordings. While we have not investigated the basis of this observation, part of the difference will result from the broader size classification range used in the perforated patch recordings, so that the smallest group includes arteries up to 700 μm in diameter, while that of the conventional whole-cell experiments includes only true 4th order branches up to 290 μm, and a small part is also due to current having been measured at a slightly less negative

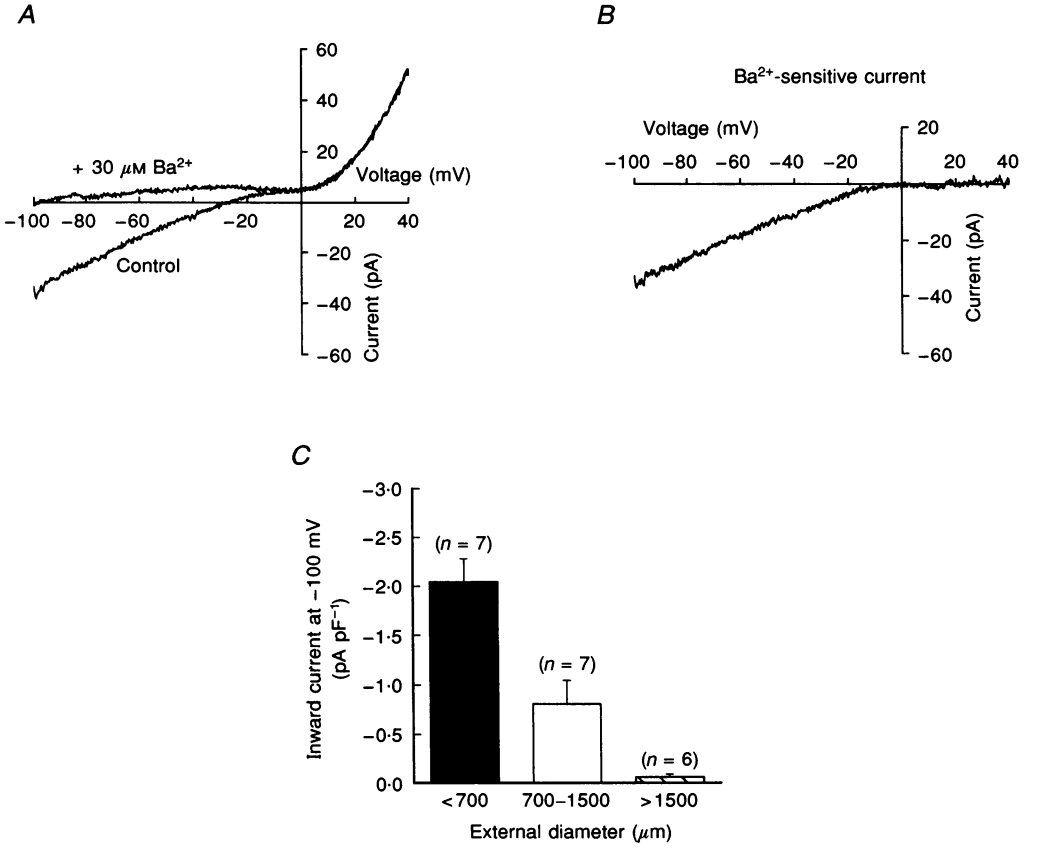

Figure 7. Potassium currents recorded in the perforated patch configuration

A, potassium current–voltage relationships in a cell from a 4th order branch of the LAD. Membrane current was recorded during a voltage ramp from −100 to +40 mV over 80 ms in control solution (Control), and after addition of 30 μM barium to the extracellular solution. Recordings were made in the perforated patch configuration of the patch clamp technique. Both $[K^+]_o$ and $[K^+]_i$ (pipette $[K^+]$) were 140 mM. Membrane currents have a positive offset due to charging of the cell capacitance during the voltage ramp and incomplete capacity compensation. *B*, barium-sensitive current in this cell obtained by subtracting current in the presence of 30 μM barium from the control recording. *C*, mean (+ S.E.M.) inward current density recorded in single smooth muscle cells at −100 mV isolated from arteries of different diameters. The number of cells is given above each histogram bar.

voltage. Other possible contributing factors could include the presence of intracellular blocking ions such as calcium, magnesium and polyamines in the perforated patch configuration. Extracellular calcium was also at a higher concentration for the perforated patch recordings (1·8 *versus* 0·1 mM), and extracellular calcium partially inhibits $I_{K(IR)}$ currents in cerebral artery cells at this concentration (J. M. Quayle & N. B. Standen, unpublished observations). Thus larger $I_{K(IR)}$currents are recorded in cells from small arteries using either conventional or perforated whole-cell patch clamp, suggesting that the dependence on arterial diameter does not arise as a consequence of the intracellular perfusion that occurs with conventional whole-cell recording.

Voltage-dependent potassium currents in pig coronary arteries

Although we used the same isolation procedure and time to obtain cells from small and large arteries, we have considered the possibility that $I_{K(IR)}$ currents were smaller in cells from large arteries because the cells suffered more damage during isolation. If that were so, we might expect currents through different types of channel to also be smaller in cells from larger arteries. We therefore also examined the properties of voltage-activated outward potassium currents ($I_{K(V)}$) in coronary artery myocytes. Depolarizing voltage steps from a holding potential of −70 mV in a physiological potassium gradient (6 mM K_o^+, 140 mM K_i^+) activated outward potassium currents (Fig. 8). This potassium current probably passes predominantly through voltage-dependent potassium channels as solutions were designed to minimize the contribution of calcium-activated potassium channels (see Methods). The current was potassium selective, and activated and inactivated on membrane depolarization (data not shown).

$I_{K(V)}$, measured at +30 mV and normalized to cell capacitance, is plotted against the external diameter of the

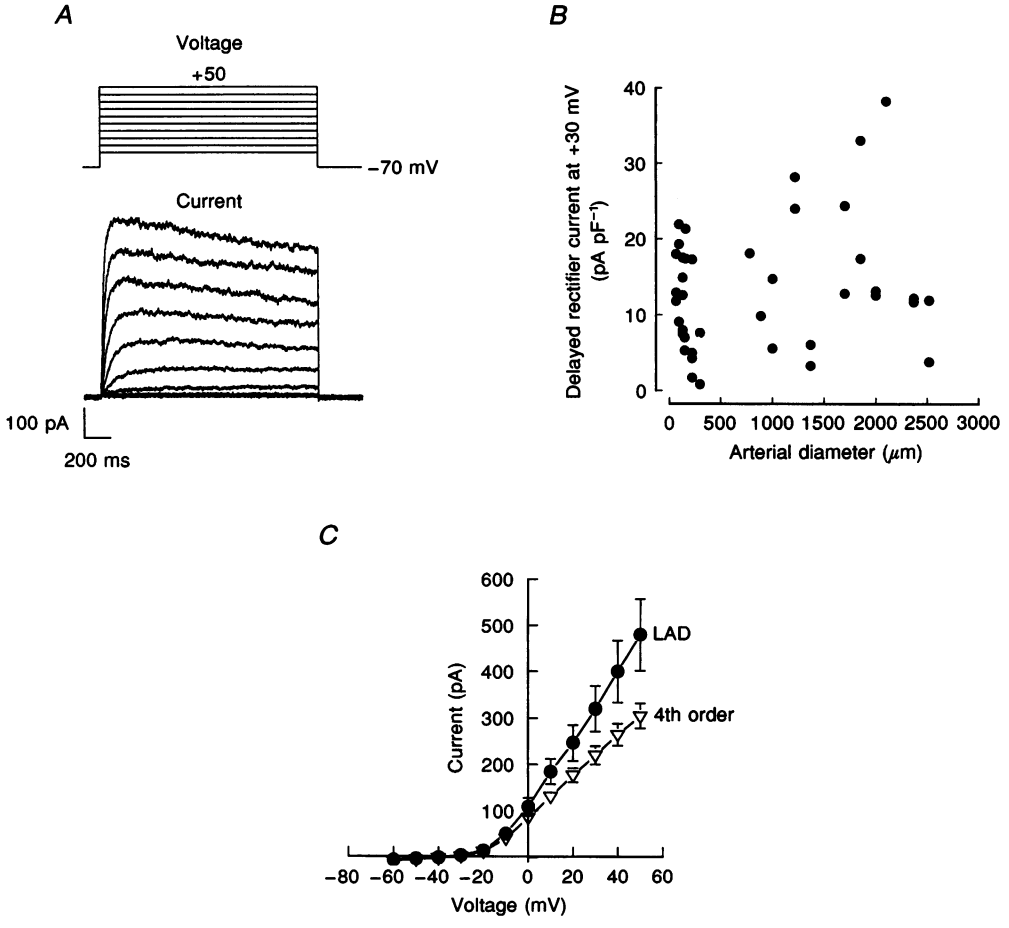

Figure 8. Voltage-dependent potassium currents in coronary artery smooth muscle cells

A, current recorded in response to depolarizing voltage steps from a holding potential of −70 mV to a test potential between −60 and +40 mV in 10 mV increments. $[K^+]_o$, 6 mM; $[K^+]_i$, 140 mM. *B*, density of outward potassium ($I_{K(V)}$) current, measured at +30 mV and 320 ms after the start of the depolarizing step, and normalized to cell capacitance, as a function of the external diameter of the artery from which single smooth muscle cells were isolated. Each symbol represents an individual cell. *C*, mean current–voltage relationships for $I_{K(V)}$ recorded in cells from the LAD (●) and a 4th order branch of this artery (▽). The symbols (●,▽) show mean values ± S.E.M. from 10 or 11 and 15 cells, respectively.

artery from which the cell was isolated in Fig. 8*B*. Unlike $I_{K(IR)}$, the density of $I_{K(V)}$ did not vary consistently with arterial diameter. Mean current density appeared slightly larger in cells from larger arteries, but this difference was not statistically significant. For example, $I_{K(V)}$ measured at +30 mV was $12{\cdot}8 \pm 1{\cdot}3$ pA pF^{-1} (19 cells) in 4th order branches and $17{\cdot}4 \pm 3{\cdot}1$ pA pF^{-1} (11 cells) in the LAD ($P = 0{\cdot}13$, t test). The mean current–voltage relationships for $I_{K(V)}$ in the LAD and 4th order branches of this artery are plotted in Fig. 8*C*.

DISCUSSION

Comparison of the arterial inward rectifier potassium current with other inward rectifiers

The properties and distribution of ion channels in vascular smooth muscle cells have a considerable influence on the regulation of arterial diameter and therefore blood flow (Nelson & Quayle, 1995). In this study we show that single cells isolated from pig coronary arteries have inward rectifier potassium currents, and that these currents are present at greater density in small coronary arteries. Inward rectifier potassium currents have been studied in a wide variety of cell types, including cardiac muscle and skeletal muscle (e.g. Leech & Stanfield, 1981; Matsuda, 1988; Ishihara, Mitsuiye, Noma & Takano, 1989). More recently, K_{IR} channels expressed from genes encoding members of the inward rectifier potassium channel family have been studied (e.g. Kubo, Baldwin, Jan & Jan, 1993; Stanfield *et al.* 1994; Doupnik, Davidson & Lester, 1995). This channel family is characterized by subunits that contain two presumed membrane-spanning α-helices and by currents that display inward rectification, though this may be either strong or relatively weak. This channel family has recently been classified as the Kir family by Doupnik *et al.* (1995), and within this the Kir2.0 subfamily expresses channels that show strong inward rectification, a conductance which depends on the extracellular potassium concentration and on the driving force on potassium ions ($V - E_K$), and a voltage- and time-dependent gating process revealed by membrane hyperpolarization. Thus the properties of the Kir2.0 family closely resemble those of native strong inward rectifiers (K_{IR} channels) described in several tissues. Previous studies have broadly confirmed that $I_{K(IR)}$ of smooth muscle are comparable to $I_{K(IR)}$ in other cell types, though it has been suggested that there may be some detailed differences in arterioles (Edwards & Hirst, 1988). Thus the conductance has been shown qualitatively to depend both on membrane potential and $[K^+]_o$ (Edwards & Hirst, 1988; Edwards *et al.* 1988; Quayle *et al.* 1993). In addition, the smooth muscle $I_{K(IR)}$ is sensitive to inhibition by extracellular barium ions, as in other cells (Quayle *et al.* 1993).

The present study allows a more quantitative comparison between the K_{IR} channel in smooth muscle and that present in other cells, and shows that this channel is strikingly similar to native inward rectifiers of skeletal and cardiac muscle, and to cloned channels of the Kir2.0 subfamily. Thus the chord conductance for $I_{K(IR)}$ currents of coronary arteries increased e-fold for an approximately 7·4 mV hyperpolarization. Comparable steepness factors for $I_{K(IR)}$ currents are 11·9 for the channel encoded by the Kir2.1 (IRK1) gene (Stanfield *et al.* 1994), 7·5 in frog skeletal muscle (Leech & Stanfield, 1981), and 5·9 in cardiac Purkinje cells (Oliva, Cohen & Pennefather, 1990). $I_{K(IR)}$ currents in coronary arteries also showed time-dependent activation in response to a hyperpolarizing voltage step (Fig. 5), similar to that seen in cardiac and skeletal muscle, and in the cloned Kir2.1 channel (Leech & Stanfield, 1981; Ishihara *et al.* 1989; Stanfield *et al.* 1994). This activation has been attributed to an intrinsic voltage-dependent gating process or, more recently, to relief of internal polyamine block of the channel (Hagiwara & Yoshii, 1979; Ishihara *et al.* 1989; Stanfield *et al.* 1994; Lopatin, Makhina & Nichols, 1994; Fakler *et al.* 1994). The time-dependent activation of the current in coronary arteries was well fitted with an exponential function, implying first-order kinetics. The time constant was about 1·6 ms at a voltage 20 mV negative to E_K (Fig. 5). Time constants for other channels at a comparable voltage were between 1 and 2 ms for the Kir2.1 gene product (Stanfield *et al.* 1994), 14 ms in frog skeletal muscle (Leech & Stanfield, 1981), and 2 ms in ventricular myocytes (Ishihara *et al.* 1989). In coronary smooth muscle this time constant decreased e-fold for a 16·7 mV hyperpolarization compared with 24·1 mV for the IRK1 gene product, 18 mV for frog skeletal muscle, and 28·8 mV for cardiac ventricular cells.

Thus we conclude that K_{IR} channels of coronary arterial smooth muscle are very similar to other strong inward rectifiers, while similarities to the channels of the Kir2.0 subfamily suggest that members of this gene family are likely to be cloned from smooth muscle in the near future.

Distribution of inward rectifier currents in arteries

Inward rectifier potassium currents are at greater density in cells isolated from smaller coronary arteries when compared with cells from larger arteries (Figs 6 and 7). $I_{K(IR)}$ currents have not been identified in previous studies of large diameter coronary arteries, or in large arteries from a variety of other vascular beds (e.g. Dart & Standen, 1993; Xu & Lee, 1994; Nelson & Quayle, 1995). The K_{IR} channel may therefore be preferentially located in small arteries in other vascular beds as well as the coronary circulation. As outlined in the Introduction, small arteries have distinct properties and physiological roles, and the location of $I_{K(IR)}$ currents in small arteries is likely to have consequences for these vessels (see below). The origin of this difference in channel density is unknown. However, factors such as the pattern of innervation, the level of myogenic tone and the presence of local metabolic vasodilators will differ between large and small arteries, and could all influence channel expression (Jones *et al.* 1995).

Voltage-activated potassium current density is comparable in small and large arteries, although large arteries appeared to have a somewhat higher current density (Fig. 8). This would argue against the possibility that the observed difference in $I_{K(IR)}$ current density was a function of cell isolation or current recording conditions. Although it is possible that the $I_{K(IR)}$ current may be more susceptible to these factors than the $I_{K(V)}$ current, other data suggest that the K_{IR} channel is relatively insensitive to cell dialysis. For example, although conventional whole-cell recordings were carried out in the absence of MgATP, no run-down in channel activity was seen during our recordings (authors' unpublished observations). Although the difference in $I_{K(V)}$ currents seen between arteries of different diameter was relatively small we cannot exclude more subtle changes, e.g. a change in the density of particular subclasses of K_V channels.

The possible physiological role of inward rectifier potassium channels in coronary arteries

The K_{IR} channel is likely to be involved in the regulation of the cell membrane potential in coronary resistance arteries. Activation of this channel has been proposed to underlie potassium-induced vasodilatations in small coronary and cerebral arteries (Edwards *et al.* 1988; McCarron & Halpern, 1990; Knot, Zimmermann & Nelson, 1994). Potassium-induced vasodilatation may be involved in the metabolic regulation of local coronary blood flow and in potassium homeostasis in the heart (see Introduction and Olsson & Bünger, 1987; Wilde & Asknes, 1995). Large diameter arteries, which show a low density of $I_{K(IR)}$ current, do not regulate local blood flow and therefore do not need to be responsive to local metabolites such as potassium ions (Jones *et al.* 1995). Control of ion channel expression may provide a means of regulating the responsiveness of arteries of different diameter and therefore function.

Potassium ions are likely to be one of several physiologically important local metabolic vasodilators, and have been suggested to be relevant in both the coronary and cerebral circulations (Olsson & Bünger, 1987; Edwards *et al.* 1988). Our investigation of the effect of $[K^+]_o$ on $I_{K(IR)}$ currents provides a plausible hypothesis as to how potassium-induced dilatations may occur. Increasing $[K^+]_o$ can augment outward potassium currents through K_{IR} channels, because of the potassium-dependent gating of this channel (Figs 2–5). At physiological pressures, arterial smooth muscle cells have membrane potentials in the range −50 to −40 mV, considerably positive to E_K. Therefore if $[K^+]_o$ is elevated within a moderate range, up to about 15 mM, E_K remains negative to the resting potential (E_K will be about −56·5 mV in 15 mM K^+), and the shift in the conductance–voltage relationship of the inward rectifier, as seen in Figs 3 and 4, should result in a increase in outward potassium current through this channel. Indeed, such an increase in outward current can be seen for an increase in $[K^+]_o$ from 6 to 12 mM in Fig. 4*B*, and would be expected to cause membrane hyperpolarization. This membrane hyperpolarization should cause closure of voltage-dependent calcium channels and relaxation of the artery (Nelson *et al.* 1990; Nelson & Quayle, 1995). Increases in $[K^+]_o$ in the range up to 15 mM have been shown to cause hyperpolarization and relaxation of pressurized cerebral and coronary arteries (McCarron & Halpern, 1990; Knot *et al.* 1994). Because of the very high input resistance of arterial smooth muscle cells (see, e.g. Nelson *et al.* 1990), the currents involved would need only to be in the order of a few picoamps, but the mechanism we propose would of course rely on K_{IR} channels making a significant contribution to the resting potassium conductance of smooth muscle cells of small arteries under physiological conditions.

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